Cerebrospinal Fluid Dehydroepiandrosterone Levels Are Correlated with Brain Dehydroepiandrosterone Levels, Elevated in Alzheimer's Disease, and Related to Neuropathological Disease Stage

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Objective: It is currently unknown whether cerebrospinal fluid (CSF) neurosteroid levels are related to brain neurosteroid levels in humans. CSF and brain dehydroepiandrosterone (DHEA) levels are elevated in patients with Alzheimer's disease (AD), but it is unclear whether CSF DHEA levels are correlated with brain DHEA levels within the same subject cohort. We therefore determined DHEA and pregnenolone levels in AD patients (n = 25) and cognitively intact control subjects (n = 16) in both CSF and temporal cortex.

Design: DHEA and pregnenolone levels were determined by gas chromatography/mass spectrometry preceded by HPLC. Frozen CSF and temporal cortex specimens were provided by the Alzheimer's Disease Research Center at Duke University Medical Center. Data were analyzed by Mann-Whitney *U* test statistic and Spearman correlational analyses.

Results: CSF DHEA levels are positively correlated with temporal cortex DHEA levels ($r = 0.59$, $P < 0.0001$) and neuropathological disease stage (Braak and Braak) ($r = 0.42$, $P = 0.007$). CSF pregnenolone levels are also positively correlated with temporal cortex pregnenolone levels ($r = 0.57$, $P < 0.0001$) and tend to be correlated with neuropathological disease stage (Braak) ($r = 0.30$, $P = 0.06$). CSF DHEA levels are elevated ($P = 0.032$), and pregnenolone levels tend to be elevated ($P = 0.10$) in patients with AD, compared with cognitively intact control subjects.

Conclusions: These findings indicate that CSF DHEA and pregnenolone levels are correlated with temporal cortex brain levels of these neurosteroids and that CSF DHEA is elevated in AD and related to neuropathological disease stage. Neurosteroids may thus be relevant to the pathophysiology of AD. **(*J Clin Endocrinol Metab* 93: 3173–3178, 2008)**

We have previously determined that dehydroepiandrosterone (DHEA) levels are elevated in postmortem prefrontal cortex (1) and temporal cortex (2) in patients with Alzheimer's disease (AD), compared with cognitively intact control subjects, and positively correlated with neuropathological disease stage (Braak and Braak) in both brain regions (1, 2). These findings are consistent with a prior report of elevated DHEA levels in postmortem brain tissue from patients with AD, compared with control subjects (3). Although there have also been published investigations of elevated DHEA levels in cerebrospi-

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Abbreviations: AD, Alzheimer's disease; ADRC, Alzheimer's Disease Research Center; CSF, cerebrospinal fluid; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; NMDA, *N*-methyl-D-aspartate; PBR, peripheral benzodiazepine receptor; PMI, postmortem interval; PREG, pregnenolone.

nal fluid (CSF) in AD patients (3, 4), to our knowledge there have been no reports comparing CSF neurosteroid levels and brain neurosteroid levels within the same subject cohort. If CSF neurosteroid levels are correlated with brain neurosteroid levels in humans, it is possible that the identification and quantification of these molecules in accessible tissues such as CSF could have utility in the early identification and diagnosis of AD. We therefore determined DHEA and pregnenolone (PREG) levels in CSF from patients with AD (n = 25) and cognitively intact control subjects (n = 16) for whom brain tissue (temporal cortex) was also available and determined whether CSF neurosteroid levels are correlated with temporal cortex neurosteroid levels within this subject cohort. Because DHEA and PREG enhance cognitive performance (5) and demonstrate neuroprotective effects in a number of rodent models (6, 7), these molecules may be relevant to the pathophysiology and treatment of AD. We determined previously that serum PREG levels are strongly correlated with brain PREG levels in rodents (8), and therefore hypothesize that a similar relationship may also be present between CSF and temporal cortex neurosteroid levels in humans.

Subjects and Methods

Postmortem CSF samples

Postmortem CSF was available for 41 (25 AD and 16 cognitively intact control subjects) of 81 subjects (40 AD and 41 cognitively intact control subjects) whose temporal cortex tissue samples had been analyzed and reported previously (2). CSF and temporal cortex tissue were generously provided by the Joseph and Kathleen Bryan Alzheimer's Disease Research Center (ADRC) at Duke University Medical Center and analyzed for DHEA and PREG. Temporal lobe boundaries were the superior and middle temporal gyri. Because brain tissue in this ADRC collection has also been used for other studies, the rostral-caudal location varied to some degree. Subjects were enrolled in the ADRC autopsy and brain donation program, as described previously (9). Procedures for enrollment were approved by the Duke University Medical Center Institutional Review Board. Cognitively intact control subjects had no neurological disorders and died of natural causes owing to advanced age. AD was diagnosed clinically according to National Institute of Neurological and Communicative Disorders/Alzheimer's Disease and Related Disorders Association criteria and was confirmed at autopsy with the National Institute on Aging/Reagan Institute criteria. Neuropathological disease stage was determined with the Braak and Braak method (10).

Gas chromatography/mass spectrometry analyses

Neurosteroid analyses were performed by a highly sensitive and specific gas chromatography/mass spectrometry method preceded by HPLC purification, as previously described (1). CSF was homogenized in 5 volumes of distilled water containing a trace quantity (4000 dpm) of tritiated neurosteroid (NEN Life Science Products, Wellesley, MA) to detect the HPLC fraction of interest as well as a constant amount of deuterated PREG (D4-PREG, 400 pg) as the internal standard (Cambridge Isotopes, Andover, MA). Supernatants were extracted three times with three volumes of ethyl acetate and dried under nitrogen before HPLC. Each steroid was collected into a separate fraction on the basis of the retention time of its radioactive analog, using hexane, tetrahydrofuran, and ethanol in the mobile phase. Samples were then transferred to 1 ml Reacti-Vials (Pierce Chemical, Rockford, IL), evaporated to dryness, and derivatized with heptafluorobutyric acid anhydride. Standards and samples were injected onto an Agilent 5973 mass spectrometer coupled to a 6890N gas chromatograph (Agilent, Santa Clara, CA) and analyzed in the negative ion chemical ionization mode with methane as the reaction gas and helium as the carrier gas. Samples were injected in duplicate. The mean intraassay coefficient of variation was 5.1% for DHEA and 6.2% for PREG. The limit of detection of this method was 2 pg for DHEA and 5 pg for PREG.

Statistical analyses

Neurosteroid levels in AD patients and cognitively intact control subjects were analyzed nonparametrically by Mann-Whitney *U* test statistic. Correlational analyses (neurosteroid levels *vs.* Braak and Braak neuropathological disease stage) were also assessed nonparametrically and Spearman correlation coefficients were determined. Both AD and control subjects were included in the correlational analyses because cognitively intact control subjects may meet neuropathological criteria for early Braak stages (potentially reflecting the earliest stages of AD or predisposition to developing AD in the absence of detectable clinical symptomatology).

Results

CSF DHEA levels are positively correlated with temporal cortex DHEA levels (Spearman r = 0.59, $P < 0.0001$, Fig. 1A). Similarly, CSF PREG levels are positively correlated with temporal cortex PREG levels (Spearman r = 0.57, $P < 0.0001$, Fig. 1B), suggesting that CSF neurosteroids may potentially serve as proxy or surrogate markers for brain neurosteroid levels. CSF DHEA levels are positively correlated with neuropathological disease stage (Braak and Braak) (Spearman r = 0.42, $P = 0.007$, Fig. 2A). CSF PREG levels tend to be positively correlated with neuropathological disease stage (Spearman r = 0.30, $P = 0.06$, Fig. 2B). CSF DHEA levels are significantly elevated in patients with AD, compared with cognitively intact control subjects (median DHEA levels 0.33 ng/ml in AD patients *vs.* 0.17 ng/ml in cognitively intact control subjects; Mann-Whitney *U* test statistic $P = 0.032$, Fig. 3A). CSF PREG levels tend to be higher in the AD group, compared with cognitively intact control subjects, but this result did not achieve statistical significance (median PREG levels 0.15 ng/ml in AD patients *vs.* 0.10 ng/ml in control subjects; Mann-Whitney *U* test statistic $P = 0.10$, Fig. 3B).

Median age for cognitively intact control subjects was 82.0 yr and median age for subjects with AD was 81.0 yr. There was no significant age difference between these two groups (Mann-Whitney, $P = 0.26$). Postmortem interval (PMI) was less than 35 h for all tissue specimens tested. Median PMI was 6.5 h for AD subjects and 7.8 h for control subjects. There was no significant difference between the median PMI of the AD group and the median PMI of the cognitively intact control groups (Mann-Whitney, $P = 0.60$). No significant correlations were found between PMI and DHEA or PREG levels in AD patients (Mann-Whitney, $P = 0.40$ and $P = 0.67$, respectively) or between PMI and DHEA or PREG levels in cognitively intact control subjects (Mann-Whitney, $P = 0.49$ and $P = 0.61$, respectively).

Discussion

This is the first report, to our knowledge, of human neurosteroid levels determined in both CSF and brain tissue within the same

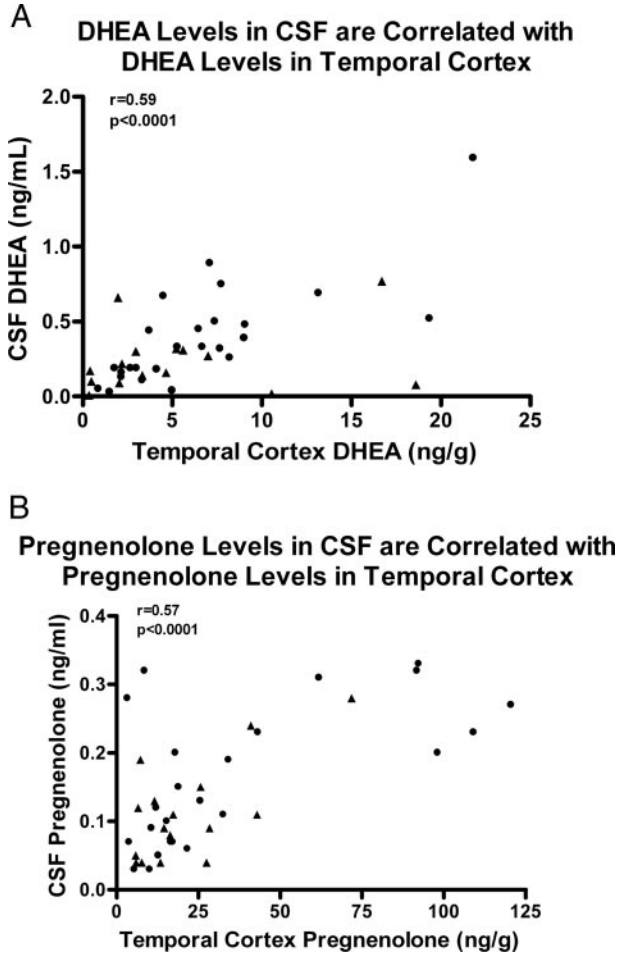

FIG. 1. A, CSF DHEA levels are correlated with temporal cortex DHEA levels (Spearman r = 0.59, $P < 0.0001$). ▲, Control; ●, AD. To ensure adequate resolution of individual data points for graphical presentation, one point (x = 40.31/y = 1.43) is not included in this figure but included in all nonparametric statistical analyses. B, CSF PREG levels and temporal cortex PREG levels are correlated (Spearman r = 0.57, $P < 0.0001$). ▲, Control; ●, AD. To ensure adequate resolution of individual data points for graphical presentation, one point (x = 276.23/y = 0.37) is not included in this figure but included in all nonparametric statistical analyses.

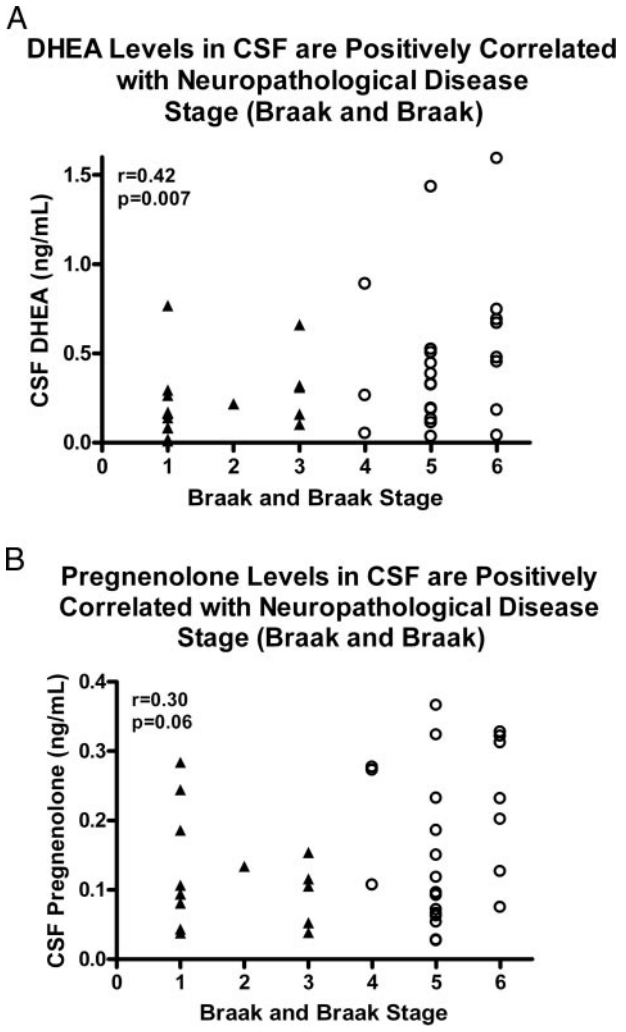

FIG. 2. A, CSF DHEA levels are positively correlated with neuropathological disease stage (Braak and Braak) (Spearman r = 0.42, $P = 0.007$). ▲, Control; ○, AD. B, CSF PREG levels tend to be positively associated with neuropathological disease stage (Braak and Braak) (Spearman r = 0.30, $P = 0.06$). ▲, Control; ○, AD.

subject cohort. Our findings indicate that CSF levels of DHEA and PREG are positively correlated with temporal cortex levels of these respective neurosteroids and that CSF DHEA is elevated in AD and positively correlated with neuropathological disease stage (Braak and Braak).

CSF and temporal cortex neurosteroid levels are correlated

Our data demonstrate a significant positive correlation between CSF DHEA and temporal cortex DHEA. CSF PREG and temporal cortex PREG are also significantly correlated. These findings therefore suggest that CSF DHEA and PREG levels reflect temporal cortex brain levels of these neurosteroids and that the determination of neurosteroid levels in CSF may have potential utility in the assessment of AD. Extensive future research will be required to replicate these findings in a larger number of subjects and test the possibility introduced by this pilot study that neurosteroid profiling may have clinical applications in AD. Because CSF DHEA and PREG levels may serve as proxy or surrogate markers reflecting brain levels of these neurosteroids, we speculate that the characterization of DHEA and PREG in CSF could be relevant to AD diagnosis.

CSF DHEA levels are associated with neuropathological disease stage

We determined in the current investigation that CSF DHEA levels are positively correlated with Braak and Braak neuropathological disease stage [similar to previously reported results in prefrontal cortex and temporal cortex (1)]. This neuropathological staging model identifies and differentiates progressive stages of AD development (10). The model proposes a predictable pattern of neuropathological progression based on neurofibrillary tangle formation. Because evidence suggests that Braak and Braak neuropathological disease stage is associated with degree of cognitive impairment (11, 12), our findings that CSF neurosteroid levels are related to neuropathological disease stage of AD may have functional significance and utility for the prediction of clinical course. However, given the relatively high degree of overlap between CSF DHEA levels in AD and control

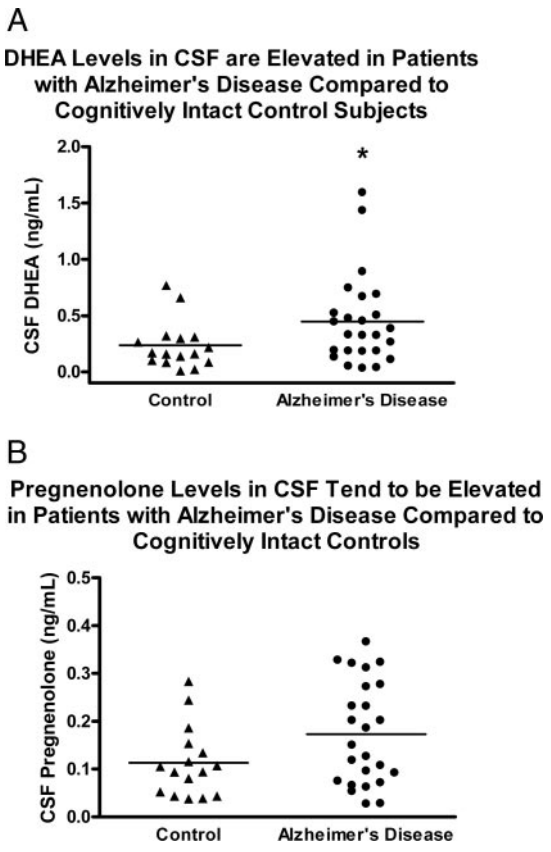

FIG. 3. A, CSF DHEA levels are elevated in patients with AD, compared with cognitively intact control subjects (0.33 ng/ml in AD *vs.* 0.17 ng/ml in control subjects; Mann Whitney *U* test statistic, $P = 0.032$). B, CSF PREG levels tend to be elevated in patients with AD, compared with cognitively intact control subjects (0.15 ng/ml in AD *vs.* 0.10 ng/ml in control subjects; Mann Whitney *U* test statistic, $P = 0.10$).

patients, it is of importance to note that CSF DHEA measurements may not be useful for the reliable detection of early stages of AD.

CSF DHEA levels are elevated in patients with AD

Our determination that CSF DHEA levels are increased in AD patients, compared with cognitively intact control subjects, is consistent with prior CSF studies (3, 4). In the current study, CSF DHEA levels in both cognitively intact control subjects and AD patients appear to be somewhat higher than previously published reports (3, 4), but the magnitude of CSF DHEA elevations in AD, compared with control subjects is similar in all three investigations (more than a doubling of CSF DHEA levels in the AD group, compared with the control group) (3, 4). In addition to elevations in DHEA in CSF, prior studies have shown increases in DHEA in a number of brain regions in AD as well (1–3).

Potential clinical ramifications of CSF DHEA elevations in AD and correlation with neuropathological disease stage

The possible etiology and functional significance of elevated DHEA levels in AD and correlation with neuropathological disease stage are not entirely clear. It may be noteworthy that levels of both DHEA (13) and PREG (14) decline with age and that there is a hypothesized role for neurosteroids in memory dysfunction (1, 5). However, it is currently not known whether CSF DHEA elevations in AD represent adaptive or nonadaptive responses and/or epiphenomena.

DHEA is neuroprotective against amyloid β-protein toxicity and attenuates β_{25-35}-amyloid peptide-induced memory impairment (15). Increases in DHEA therefore may be reflective of an adaptive or compensatory mechanism in AD. Additionally, elevated DHEA levels in the later stages of AD may be related to elevated stress experienced by more severely ill patients with AD. It is also possible that increases in DHEA in AD may result from β-amyloid deposition because β-amyloid administration increases DHEA formation in oligodendrocytes (16). Oxidative stress also plays an important role in the pathophysiology of AD (17–21), and DHEA has neuroprotective effects against various insults that result in oxidative stress. For example, DHEA is neuroprotective against anoxia (7), glucocorticoid-induced toxicity (22), $H_2O_2/FeSO_4$-stimulated lipid oxidation (23), 3-nitropropionic acid-induced oxidation stress (24), and glutamate- (25) and acute *N*-methyl-D-aspartate (NMDA)-induced excitotoxicity (26). Additionally, DHEA has neurotrophic effects and increases neurogenesis (27, 28). Moreover, in rodent models DHEA augments learning and memory (29) and may also impact episodic memory in humans (30). In contrast, a small placebo-controlled double-blind clinical trial of DHEA augmentation in AD patients was not associated with improved cognitive performance using a dose of 50 mg twice daily for 6 months (31).

It has also been hypothesized that excitotoxicity may play a role in the pathophysiology of AD (32). Because DHEA positively modulates excitatory NMDA receptors (33) and negatively modulates inhibitory γ-aminobutyric $acid_A$ receptors (34), elevated DHEA levels in AD could potentially result in a net increase in excitation and represent a nonadaptive response contributing to this aspect of AD pathophysiology.

In addition to potential effects of DHEA on pathophysiology, it is equally important to consider possible causal factors leading to DHEA elevations. The existing literature supports the possibility that some of DHEA's actions may be mediated via downstream conversion to other steroids. DHEA conversion to other steroids appears to be reduced in AD, potentially contributing to DHEA elevations. For example, DHEA metabolism to 7α-hydroxy-DHEA and androstenediol tends to be decreased in frontal cortex in subjects exhibiting increased amyloid plaque density (35), and cytochrome P450 enzyme CYP7B (which produces 7α-hydroxy-DHEA from DHEA) mRNA expression in dentate gyrus and CA1 pyramidal neurons is significantly reduced in AD (36). Future efforts will be required to determine the precise etiological mechanisms leading to DHEA alterations in patients with AD.

Previous studies also provide evidence that changes in the levels of the sulfated derivative of DHEA (DHEAS) may be important in AD pathophysiology. Our finding of increased CSF DHEA levels in AD patients is consistent with the findings of Kim *et al.* (4), who additionally reported decreased levels of CSF DHEAS in AD patients. Many roles have previously been attributed to DHEAS, including prevention and reduction of the neurotoxic effects of NMDA, 2-amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid (AMPA), and kainic acid (37), and reduction

of anxiety and improvement of memory in animals (5, 38). Thus, it is possible that reduced DHEAS levels in AD (4) may negatively impact several neuroprotective processes. Investigating both DHEA and DHEAS as well as the ratio of DHEA to DHEAS, will therefore be important in future studies focusing on the clinical relevance of these neurosteroids to the pathophysiology of AD.

Finally, AD has also been associated with alterations in the peripheral benzodiazepine receptor (PBR), which plays a major role in regulating cholesterol transport into mitochondrial membranes (39). Disruptions in cholesterol regulation could thus theoretically impact downstream neurosteroid formation. Notably, PBR binding is significantly increased in temporal cortex (40, 41) and moderately increased in frontal cortex (40) of AD patients. It therefore is possible that increases in PBR binding in AD may result in changes in steroidogenesis and impact DHEA and DHEAS levels. Additional efforts characterizing the relationship between PBR activation and downstream neurosteroid production in AD will be required to address this possibility.

Study limitations

One limitation of this study is relatively small sample size (postmortem CSF was available for 25 AD and 16 cognitively intact control subjects of 40 AD and 41 cognitively intact control subjects in the original cohort), a challenge that is typical of human postmortem tissue investigations. These findings will thus require replication in a larger sample. In addition, it was not possible to control for medication status at the time of death in either patients with AD or cognitively intact control subjects. Pharmacological agents such as the antidepressant fluoxetine (42, 43) and the antipsychotics olanzapine and clozapine (8) have been shown to increase PREG levels in rodent models. Fluoxetine may also enhance activity of a neurosteroidogenic enzyme (44), although this finding has not been replicated (45). Larger investigations controlling for medication use will therefore be necessary to confirm the findings of the current study. Finally, information regarding smoking is not available for this cohort, a variable that may influence neurosteroid levels (46, 47).

Summary

In summary, our findings demonstrate that CSF DHEA and PREG levels are positively correlated with temporal cortex levels of these respective neurosteroids within the same patient cohort. CSF DHEA levels are positively correlated with neuropathological disease stage (Braak and Braak) and are elevated in AD, compared with cognitively intact control subjects. Because CSF neurosteroid levels appear to be reflective of temporal cortex levels, CSF DHEA and PREG may potentially serve as proxy or surrogate markers for brain neurosteroid levels and have utility in the diagnosis of AD and the prediction of clinical course.

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